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THE DISSOCIATION OF NUCLEAR PROTEINS FROM SUPERHELICAL DNA

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SUMMARY

Structures retaining many of the morphological features of nuclei may be released by gently lysing human cells in solutions containing non-ionic detergents and high concentrations of salt. These nucleoids contain superhelical DNA. Using a double-labelling procedure we have compared, at different salt concentrations, the amounts and types of protein associated with human nucleoids containing superhelical or relaxed DNA. We find that the slightly lysine-rich histones (H2A and H2B) but not the arginine-rich histones (H3 and H4) dissociate more slowly from nucleoids containing superhelical DNA than from those containing relaxed DNA. A protein of apparent molecular weight of 22000 also binds more tightly to superhelical DNA. We conclude that this protein and the slightly lysine-rich histones transmute free energy of supercoiling into binding energy when they bind to superhelical DNA.

INTRODUCTION

One description of the folding of the circular DNA of viruses is based upon a distinction between the right-handed helical turns of the double helix and any superhelical turns that might be superimposed upon the duplex turns (Bauer & Vinograd, 1968, 1971). We have been studying DNA conformation in nuclear structures derived by lysing cells in solutions containing non-ionic detergents and high concentrations of salt. These nucleoids contain all the nuclear RNA and DNA (Colman & Cook, 1977). The nucleoid DNA is supercoiled and the supercoiling may be released by breaking the DNA with γ-rays (Cook & Brazell, 1975, 1976*a*, *b*, 1977); (see also Ide, Nakane, Anzai & Andoh, 1975; Benyajati & Worcel, 1976). Nucleoids isolated in high salt concentrations contain few of the proteins characteristic of chromatin; when isolated in lower salt concentrations they contain histones (Cook, Brazell & Jost, 1976). Recent evidence suggests that chromatin is composed of subunits (Kornberg, 1974; Senior, Olins & Olins, 1975; Griffith, 1975) and histones H3 and H4 have been implicated in the primary folding of DNA in the nucleus (Wilkins, Zubay & Wilson, 1959; Richards & Pardon, 1970; Boseley *et al.* 1976; Camerino-Otero, Solner-Webb & Felsenfeld, 1976). Using a double-labelling procedure, we have compared the amounts and types of protein bound to human nucleoids containing superhelical or relaxed DNA. The comparison indicates that the slightly lysine-rich histones (H2A and H2B), but not the arginine-rich histones (H3 and H4), remain bound more tightly to superhelical DNA than to relaxed DNA.

METHODS AND MATERIALS

Cells and radioactive labelling

HeLa cells were grown in suspension as described (Cook & Brazell, 1975). Two batches of cells were labelled simultaneously for 20–24 h, one with [^{3}H]leucine (The Radiochemical Centre, Amersham; 100 μCi/ml; 54000 Ci/mol), the other with [^{14}C]leucine (50 μCi/ml; 330 Ci/mol). Cells grown in [^{14}C]leucine multiplied slightly more slowly than in [^{3}H]leucine. Labelled cells were washed 3 times in phosphate-buffered saline (Dulbecco & Vogt, 1954) and resuspended in phosphate-buffered saline at room temperature.

Mixtures used for lysing cells

A number of mixtures, adjusted to pH 8·0 at 20 °C but containing different salt concentrations, were used to lyse cells. The final salt concentration of the mixture after the addition of cells is designated in parentheses. We neglect the contribution of the phosphate-buffered saline in calculating the final concentration of sodium chloride. Lysis mixtures (1·95 M NaCl), (1·2 M NaCl), (1·0 M NaCl) and (0·75 M NaCl) all contained EDTA, Tris and Triton such that after adding 1 vol. of phosphate-buffered saline containing cells to 3 vol. of the mixture, the final concentrations of the constituents become 0·1 M, 2 mM and 0·5 % respectively. Lysis mixture (0·4 M NaCl) contained EDTA, Tris, Brij and glycerol such that on addition of 1 vol. of phosphate-buffered saline containing cells to 3 vol. of the mixture, the final concentration of the constituents become 0·1 M, 2 mM, 1 % and 10 % respectively.

γ-irradiation

Sucrose gradients were irradiated immediately after addition of the cells in phosphate-buffered saline to the lysis mixture floating on top of the gradients (Cook & Brazell, 1975, 1976*a*).

Sucrose and glycerol gradients

Nucleoids were isolated free of dissociated proteins after sedimentation through 'isokinetic' sucrose or glycerol gradients (Cook *et al.* 1976). Sucrose gradients (15–30 % sucrose, 4·6 ml, pH 8·0) contained sodium chloride (0·4, 0·75, 1·0, 1·2 or 1·95 M), Tris (10 mM) and EDTA (1·0 mM). Glycerol gradients (30–50 % glycerol, 4·6 ml, pH 8·0) contained NaCl (0·4 M), Tris (10 mM) and EDTA (1·0 mM). 150 μl of a lysis mixture containing the same NaCl concentration as the gradient were layered on the gradient, followed by 50 μl of phosphate-buffered saline containing cells (12–16 $\times$ 10^6 cells/ml). Sometimes ethidium at a final concentration of 4 or 16 μg/ml was added to both lysis mixture and the gradient. 10 or 15 min after the addition of the cells to the lysis mixture, the gradients were spun at 20 °C in an SW 50.1 rotor on a Beckman L2-65b ultracentrifuge at speeds and times indicated in the legends to the figures. The visible aggregates of nucleoids were collected using a 19 G needle inserted through the side of the tube.

Two gradients were generally prepared together. Where appropriate the nucleoids from each gradient were mixed immediately after isolation and the salt concentration lowered to 0·2 M by dilution in ice-cold 10 mM Tris (pH 8·0) containing 0·1 mM phenylmethylsulphonylfluoride. The mixed nucleoids were pelleted by centrifugation (Beckman SW 50.1 rotor; 40000 rev/min; 15 min; 4 °C) and stored at −70 °C.

Analysis of protein

Proteins were separated by electrophoresis on polyacrylamide gel slabs containing sodium dodecyl sulphate (Laemli, 1970). Purified histone fractions (a gift from Dr E. W. Johns) and bovine plasma albumin (Armour Pharmaceutical Co. Ltd., Eastbourne) were used as references. Nucleoid proteins were isolated by resuspending nucleoids in 1 % sodium dodecyl sulphate, 10 mM Tris (pH 8) and 1 mM phenylmethylsulphonylfluoride. After the DNA had been

pelleted by centrifugation (Beckman SW 50.1 rotor; 40000 rev/min; 3 h; 4 °C) the supernatant was dialysed against 3 changes of distilled water containing 0·1 mM phenylmethylsulphonylfluoride and then freeze-dried. The proteins were redissolved in sample buffer containing 10 % glycerol, 2 % sodium dodecyl sulphate and 0·1 M dithiothreitol. Nucleoid protein was also prepared for electrophoresis by dissolving nucleoid pellets in 75 μl sample buffer and shearing the DNA by passage through a micropipette. Mixtures of protein and sheared DNA gave identical electrophoretic patterns to those given by protein free of DNA. The gels were stained with 0·1 % Coomassie blue and photographed. Photographic negatives were analysed using a Joyce–Loebl densitometer.

Radioactive profiles of gels were obtained by slicing the gels (slices 1 mm thick) and measuring the radioactive content of each slice (Cook *et al.* 1976).

The radioactivity due to ^{3}H or ^{14}C in each slice was expressed as a fraction of the total amount of ^{3}H or ^{14}C in the whole gel. In each slice the ratio of the fractions of each label provides a sensitive index of the relative proportions of that label bound to irradiated and unirradiated nucleoids. Thus

$$\left[\frac{a_x}{\sum_n^1 a}\right] \div \left[\frac{b_x}{\sum_n^1 b}\right] = \text{affinity index}$$

where a_x and b_x are the amounts of label derived from unirradiated and irradiated nucleoids respectively in the xth slice of a gel sliced into n slices. The fraction of label from unirradiated nucleoids is the numerator so that indices above unity characterize proteins present in greater quantities in unirradiated nucleoids. Where proteins from unirradiated nucleoids isolated in the presence or absence of ethidium are compared, the fraction of label from the nucleoids isolated in the absence of ethidium is the numerator. Again indices above unity characterize proteins present in greater quantities in nucleoids whose DNA is naturally supercoiled.

RESULTS

The protein content of equal numbers of nucleoids isolated in different salt concentrations was analysed in polyacrylamide gels containing sodium dodecyl sulphate (Fig. 1). Even in 1·95 M NaCl some proteins (molecular weights > 38000) remain associated with the nucleoids (Fig. 1). Since the binding of these proteins is unaffected by radiation or ethidium (see later) they serve as useful reference proteins for comparison with the other nucleoid proteins. Nucleoids isolated in 0·4 M NaCl contain traces of histone H1 and a number of proteins which are absent in nucleoids isolated at higher salt concentrations. As the salt concentration is raised above 0·75 M NaCl, first histones H2A, H2B, and then H3 and H4 are lost from nucleoids, as is the case with chromatin (Ohlenbusch, Olivera, Tuan & Davidson, 1967; Henson & Walker, 1970); in 1·95 M NaCl the nucleoids contain no histones. A protein which we provisionally call supercoil-binding protein 1 (SBP1), of apparent molecular weight 22000, is found in nucleoids isolated at or below salt concentrations of 1·0 M NaCl.

We next compared the proteins associated with nucleoids containing DNA of different conformation. The various nucleoid proteins dissociate at different salt concentrations so that differences in the relative amounts of these proteins will only be detectable under dissociating conditions. We therefore performed 2 kinds of experiment at a variety of salt concentrations. In the first, the proteins of nucleoids with the natural DNA conformation were compared with those of nucleoids irradiated with a low dose of γ-rays sufficient to remove supercoiling but insufficient to introduce many breaks into DNA.

HeLa cells labelled with [^{3}H]leucine were lysed on top of one sucrose gradient and cells labelled with [^{14}C]leucine on top of another. One sucrose gradient was γ-irradiated. The nucleoids in both gradients were separated from dissociated protein by centrifugation and then removed from each of the 2 gradients, mixed and the

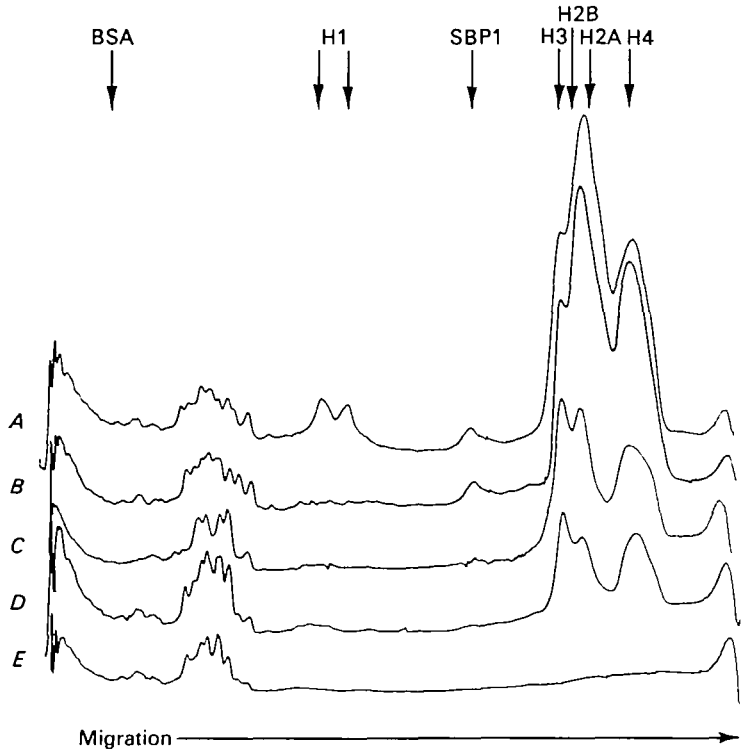

Fig. 1. A comparison of the proteins of equal numbers of nucleoids isolated at different salt concentrations. Proteins from nucleoids isolated at various salt concentrations in the absence of ethidium were separated in a polyacrylamide gel (length 10 cm) containing sodium dodecyl sulphate, stained with Coomassie blue and the optical density profile of the gel determined. The nucleoids were prepared from cells grown in [*Me*-^{3}H]thymidine (56000 Ci/mol; 0·02 μCi/ml) for 24 h and equivalent amounts of radioactivity were applied to each gel. Reference proteins, bovine plasma albumin (BSA) and histones (H1, H2A, H2B, H3 and H4) were run in adjacent channels. Proteins from nucleoids isolated in: *A*, 0·4 M NaCl; *B*, 0·75 M NaCl; *C*, 1·0 M NaCl; *D*, 1·2 M NaCl; and *E*, 1·95 M NaCl were analysed in this way.

mixture subjected to electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate. The relative proportions of ^{3}H and ^{14}C in the gel reflect the relative proportions of protein derived from irradiated or unirradiated nucleoids. We derive the affinity index – a measure of the binding to superhelical DNA relative to the relaxed form – by normalizing the radioactivities (see Materials and methods); indices above unity characterize proteins present in greater amounts in unirradiated nucleoids than irradiated nucleoids.

In the second kind of experiment the proteins of nucleoids with the natural DNA

conformation were compared with those of unirradiated nucleoids isolated in the presence of ethidium. Ethidium intercalation unwinds the double helix removing (negative) superhelical turns (e.g. in 1·0 M NaCl, 4 μg/ml ethidium removes supercoiling from nucleoid DNA). At higher concentrations (i.e. 16 μg/ml in 1·0 M NaCl) supercoils of the opposite sense to those intitially present are induced (Cook & Brazell, 1975). Cells labelled with [^{3}H]leucine were layered on one sucrose gradient and ^{14}C-labelled cells on another; neither gradient was irradiated but one contained ethidium. After the 2 kinds of nucleoids had been isolated and mixed, their proteins were subjected to electrophoresis in gels. Again affinity indices above unity characterize proteins remaining more tightly bound to nucleoids with the natural superhelical conformation.

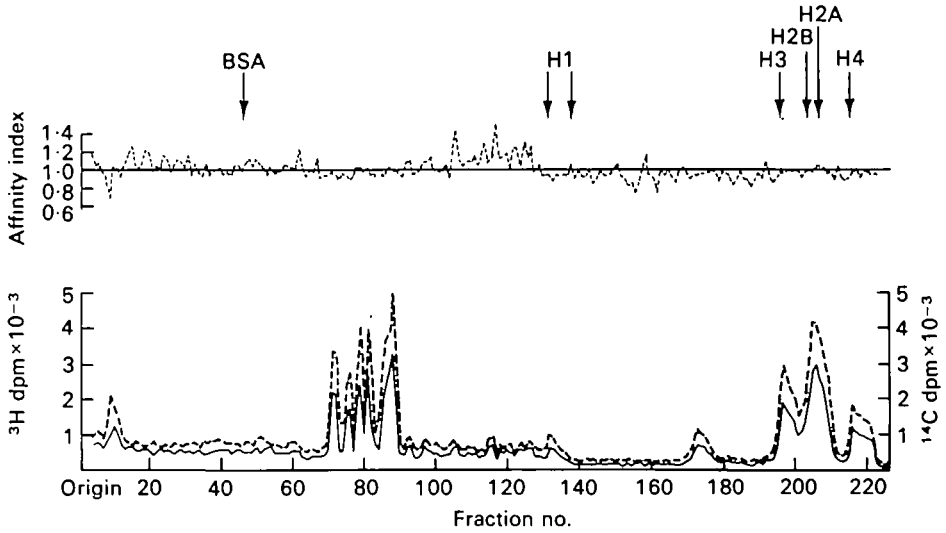

Fig. 2. A comparison of the proteins of unirradiated and γ-irradiated nucleoids isolated in 0·4 M NaCl. Cells labelled with [^{3}H]leucine or [^{14}C]leucine were applied to 2 glycerol gradients containing 0·4 M NaCl but no ethidium. The gradient containing the ^{14}C-labelled cells was γ-irradiated (9·6 J kg^{-1}; 1·2 J kg^{-1} min^{-1}). Ten min after the addition of the cells to the gradients, the gradients were spun at 5000 rev/min for 15 min. Nucleoids were collected from the 2 gradients, mixed, pelleted and the mixture subjected to electrophoresis in a polyacrylamide gel (30 cm long) containing sodium dodecyl sulphate. The gel was sliced and the radioactive content of the slices determined. Reference proteins, bovine plasma albumin (BSA) and histones (H1, H2A, H2B, H3, H4) were run in adjacent channels of the gel. — —, ^{3}H dpm from unirradiated nucleoids; ——, ^{14}C dpm from γ-irradiated nucleoids; – –, affinity index of the proteins in each slice of the gel.

Fig. 2 illustrates the protein content of irradiated and unirradiated nucleoids isolated in 0·4 M NaCl. The spectrum of proteins revealed by labelling with ^{3}H or ^{14}C resembles that identified by staining with Coomassie blue (cf. Figs. 1 and 2). The major proteins have affinity indices of about one, indicating that they are present in equal amounts in irradiated and unirradiated nucleoids. Ethidium does not alter the relative amounts of proteins of unirradiated nucleoids (Fig. 3).

Fig. 4 illustrates the proteins of nucleoids isolated in 0·75 M NaCl. The relative

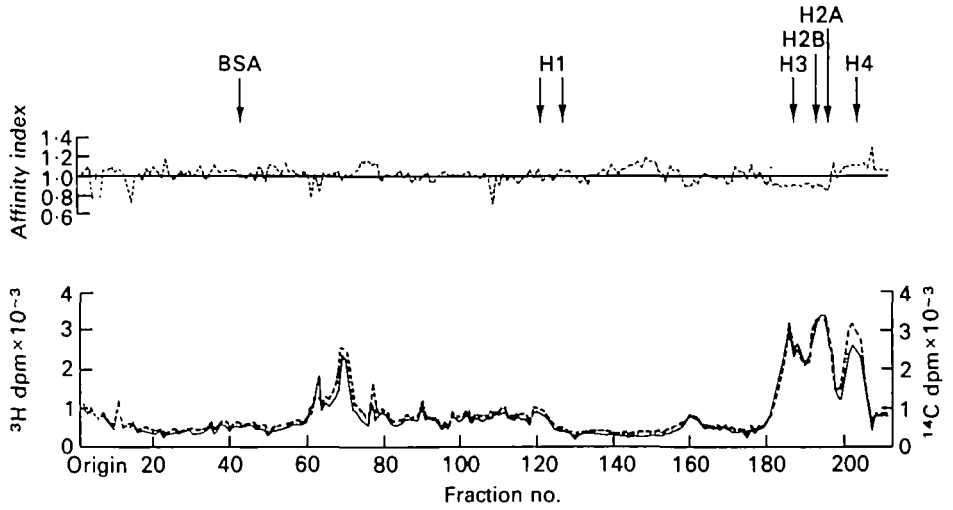

Fig. 3. The effect of ethidium on the protein content of unirradiated nucleoids isolated in 0·4 M NaCl. Cells labelled with [^{3}H]leucine were applied to a glycerol gradient containing 0·4 M NaCl but lacking ethidium. Others labelled with [^{14}C]leucine were applied to a similar gradient containing 4 μg/ml ethidium. Neither gradient was irradiated. Nucleoids were isolated and their proteins analysed, as described in the legend to Fig. 2. — —, ^{3}H dpm from nucleoids isolated in the absence of ethidium; ——, ^{14}C dpm from nucleoids isolated in the presence of ethidium; – –, affinity index of the proteins in each slice of the gel.

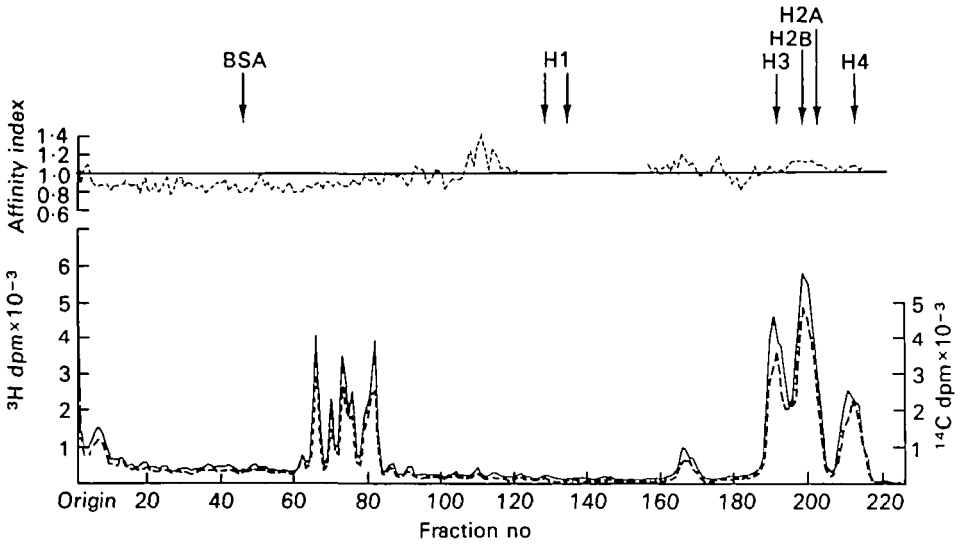

Fig. 4. A comparison of the proteins of unirradiated and γ-irradiated nucleoids isolated in 0·75 M NaCl. Cells labelled with [^{3}H]leucine or [^{14}C]leucine were applied to 2 sucrose gradients containing 0·75 M NaCl but no ethidium. The gradient containing the ^{14}C-labelled cells was γ-irradiated. 15 min after the addition of the cells to the gradients, the gradients were spun at 5000 rev/min for 12 min. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2. — —, ^{3}H dpm from unirradiated nucleoids; ——, ^{14}C dpm from irradiated nucleoids; – –, affinity index of the proteins in each slice of the gel.

amounts of proteins associated with irradiated and unirradiated nucleoids are similar; their affinity indices are about one. However, the amounts of some proteins of unirradiated nucleoids depend upon whether or not these nucleoids are isolated in the presence or absence of ethidium (Fig. 5). Nucleoids isolated in the presence of ethidium contain relatively less H2A, H2B and SBP1: the affinity indices of these proteins are much greater than one. Ethidium may drive H2A, H2B and SBP1 from nucleoids by competing for binding sites or it may alter the affinity of these 3 proteins for nucleoid DNA by altering supercoiling.

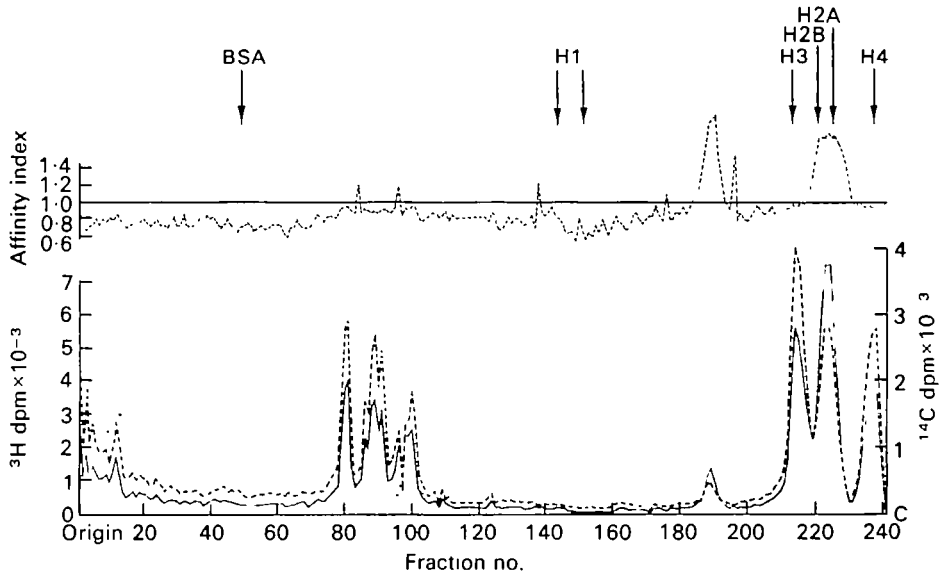

Fig. 5. The effect of ethidium on the protein content of unirradiated nucleoids isolated in 0·75 M NaCl. Cells labelled with [^{14}C]leucine were applied to a sucrose gradient containing 0·75 M NaCl but lacking ethidium. Others labelled with [^{3}H]leucine were applied to a similar gradient containing 4 μg/ml ethidium. Neither gradient was irradiated. Gradients were spun at 5000 rev/min for 12 min. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2. — —, ^{3}H dpm from nucleoids isolated in the presence of ethidium; ——, ^{14}C dpm from nucleoids isolated in the absence of ethidium; – –, affinity index of the proteins in each slice of the gel.

In 1 M NaCl SBP1, H2A and H2B dissociate from the nucleoids (Fig. 1). In the absence of ethidium more of these 3 proteins remain associated with unirradiated nucleoids than with irradiated nucleoids (Fig. 6). Four μg/ml ethidium reduces the average superhelical density of nucleoid DNA to zero: this enabled us to compare protein binding to nucleoids containing negatively supercoiled with those containing non-supercoiled DNA. Unirradiated nucleoids isolated in the presence of 4 μg/ml ethidium contain less of these 3 proteins (Fig. 7).

Histone H1 binds more tightly to both the positively and negatively supercoiled allomorphs of SV_{40} DNA than to the relaxed form (Vogel & Singer, 1975*a*, *b*). Since 16 μg/ml ethidium induces supercoiling of opposite sense to that initially present in nucleoid DNA (Cook & Brazell, 1975) we can determine whether this is also true of SBP1 and the slightly lysine-rich histones. Unirradiated nucleoids isolated in the

presence of 16 μg/ml ethidium contained less SBP1, H2A and H2B than their counterparts isolated in the absence of ethidium (Fig. 8) – none of the nucleoid proteins behave like H1. However, the ethidium used to induce positive supercoiling might compete with proteins for DNA-binding sites so preventing us from detecting any avidity for positive supercoiling; it certainly magnifies the differences in binding of H2A, H2B and SBP1.

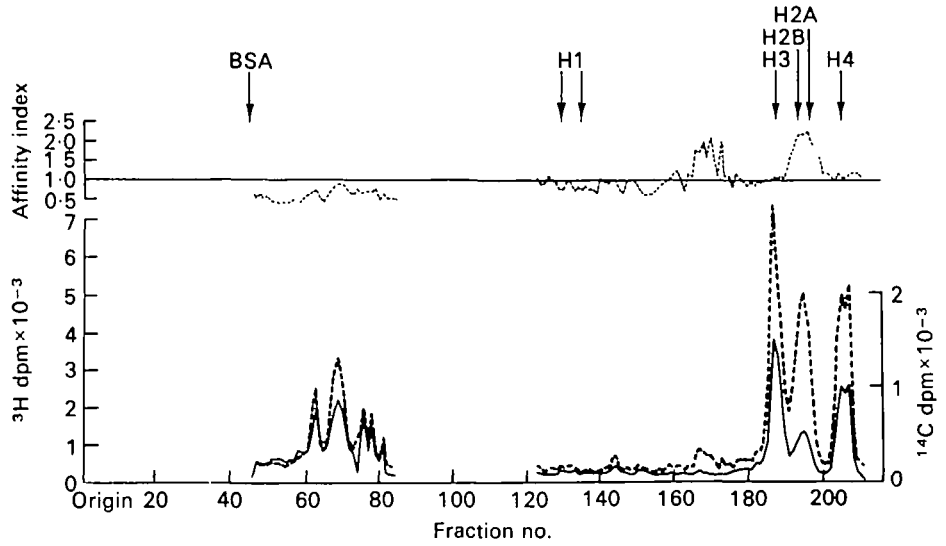

Fig. 6. A comparison of the proteins of unirradiated and γ-irradiated nucleoids isolated in 1·0 M NaCl. Cells labelled with [^{3}H]leucine or [^{14}C]leucine were applied to 2 sucrose gradients containing 1·0 M NaCl but no ethidium. The gradient containing ^{14}C-labelled cells was γ-irradiated. 15 min after the cells were layered on the gradients, the gradients were spun at 5000 rev/min for 25 min. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2; 2 parts of the gel were not analysed. — —, ^{3}H dpm from unirradiated nucleoids; ——, ^{14}C dpm from irradiated nucleoids; – –, affinity index of the proteins in each slice of the gel.

Differences in binding of SBP1, H2A and H2B are only detected under dissociating conditions (i.e. at 0·75 M and 1·0 M NaCl, but not at 0·4 M NaCl). Since little H3 and H4 dissociate from nucleoids in 1·0 M NaCl we compared the proteins of irradiated and unirradiated nucleoids isolated in 1·2 M NaCl. At this salt concentration H3 and H4 dissociate from the nucleoids and little SBP1, H2A or H2B remain (Figs. 1, 9, 10). Unirradiated and irradiated nucleoids contain similar amounts of H3 and H4 (Fig. 9): ethidium has little effect on the dissociation of these histones (Fig. 10). Even under dissociating conditions H3 and H4 do not share the higher affinity of H2A and H2B for nucleoids whose DNA is naturally supercoiled.

The differences we find in the relative proportions of ^{3}H-labelled and ^{14}C-labelled proteins cannot result from labelling artifacts. We have repeated each of the experiments described in Figs. 2–10 with the labels reversed: this does not alter the affinity indices characteristic of the separated proteins. Furthermore the major proteins in mixtures of nucleoids treated similarly in all respects except for labelling with ^{3}H or ^{14}C have affinity indices of unity (Fig. 11).

As unirradiated and irradiated nucleoids sediment different distances down the gradients, proteins dissociated in the lysis mixture might contaminate our preparations of nucleoids to different degrees. Therefore a differential contamination might underlie the differences in relative proportions of labelled proteins that we see. However, this is unlikely: extra protein is associated with the nucleoids that sediment

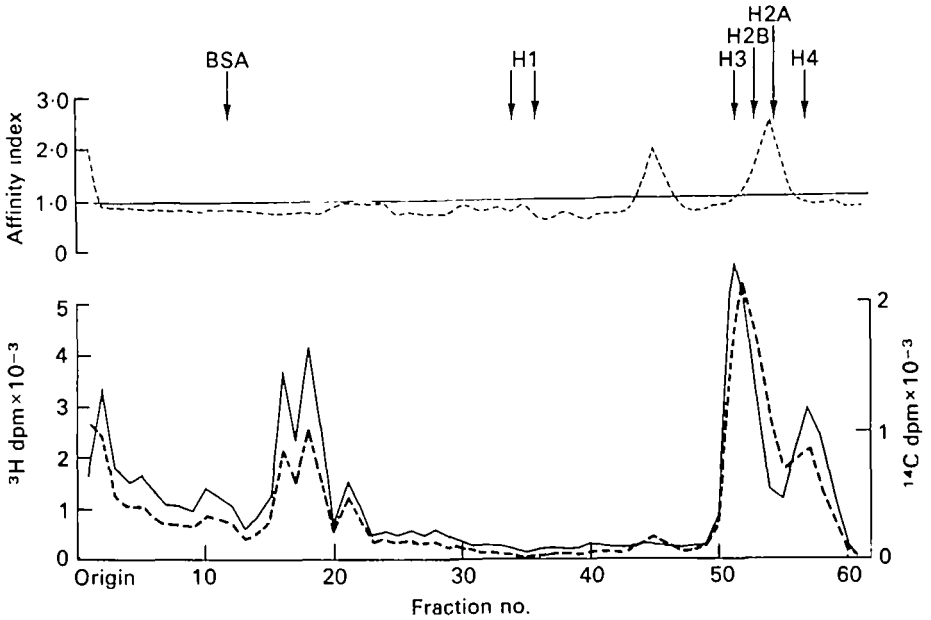

Fig. 7. The effect of ethidium on the protein content of unirradiated nucleoids isolated in 1·0 M NaCl. Cells labelled with [^{3}H]leucine were applied to a sucrose gradient containing 1·0 M NaCl but lacking ethidium. Others, labelled with [^{14}C]leucine were applied to a similar gradient containing 4 μg/ml ethidium. Neither gradient was irradiated. Gradients were spun at 5000 rev/min for 25 min. Nucleoids were isolated and their proteins analysed essentially as described in the legend to Fig. 2. A short gel (10 cm long) was used in this experiment. Histones H2A and H2B are not fully resolved. — —, ^{3}H dpm from nucleoids isolated in the absence of ethidium; ——, ^{14}C dpm from nucleoids isolated in the presence of ethidium; – –, affinity index of the proteins in each slice of the gel.

the furthest away from the lysis mixture. An additional control experiment confirms that the differences do not result from such differential contamination. Irradiated nucleoids were sedimented to the same position in gradients (containing 1·0 M NaCl) as their unirradiated counterparts by increasing the speed of centrifugation to 9000 rev/min. As before, the unirradiated nucleoids contained relatively more H2A, H2B and SBP1.

DISCUSSION

The protein SBP1 and the slightly lysine-rich histones (H2A and H2B), but not the arginine-rich histones (H3 and H4), dissociate more slowly from unirradiated nucleoids than from γ-irradiated nucleoids. Ethidium also accelerates their dissociation.

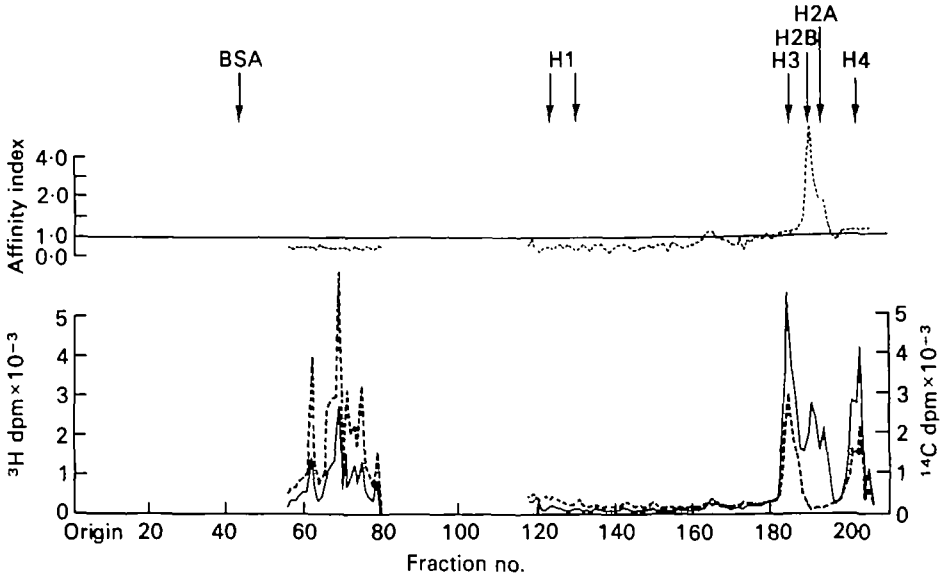

Fig. 8. The effect of ethidium on the protein content of unirradiated nucleoids isolated in 1·0 M NaCl. Cells labelled with [^{14}C]leucine were applied to a sucrose gradient containing 1·0 M NaCl but lacking ethidium. Others, labelled with [^{3}H]leucine, were applied to a similar gradient containing 16 μg/ml ethidium. Neither gradient was irradiated. Gradients were spun at 5000 rev/min for 25 min. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2; 2 parts of the gel were not analysed. — —, ^{3}H dpm from nucleoids isolated in the presence of ethidium; ——, ^{14}C dpm from nucleoids isolated in the absence of ethidium; – –, affinity index of the proteins in each gel slice.

We conclude that SBP1, H2A and H2B have a higher affinity for negatively supercoiled DNA than for DNA relaxed by irradiation or ethidium intercalation. We will argue that these histones stabilize the superhelical conformation of DNA *in vivo*.

Breaks in the nuclear DNA are readily repaired *in vivo:* repair restores the original configuration (Cook & Brazell, 1975, 1976*b*). If DNA existed *in vivo* in a high-energy superhelical state breakage of phosphodiester bonds (i.e. nicking) would allow the DNA to relax. The higher-energy configuration must then be reintroduced into the DNA during repair. We think this unlikely: instead the configuration of intact and nicked DNA in the living cell may be equally stable. Then nicking would not alter the configuration, and repair of broken phosphodiester bonds would not change the configuration. If so, the free energy of supercoiling present in nucleoid DNA in 1·0 or 1·95 M NaCl cannot be available *in vivo* to drive the relaxation of the DNA. (The free energy of supercoiling receives contributions from torsional and bending effects and from longer range interactions (Davidson, 1972; Campbell, 1976).) Both temperature and the concentration of salts will affect the degree of supercoiling so that the superhelical density of nucleoid DNA under physiological conditions will be about half that in 1·95 M NaCl at 20 °C (Cook & Brazell, 1977).

Ethidium is an agent which reduces the free energy of supercoiling when it binds to DNA. It does so by unwinding the double helix, so reducing the number of left-handed (negative) superhelical turns and diminishing free energy of supercoiling.

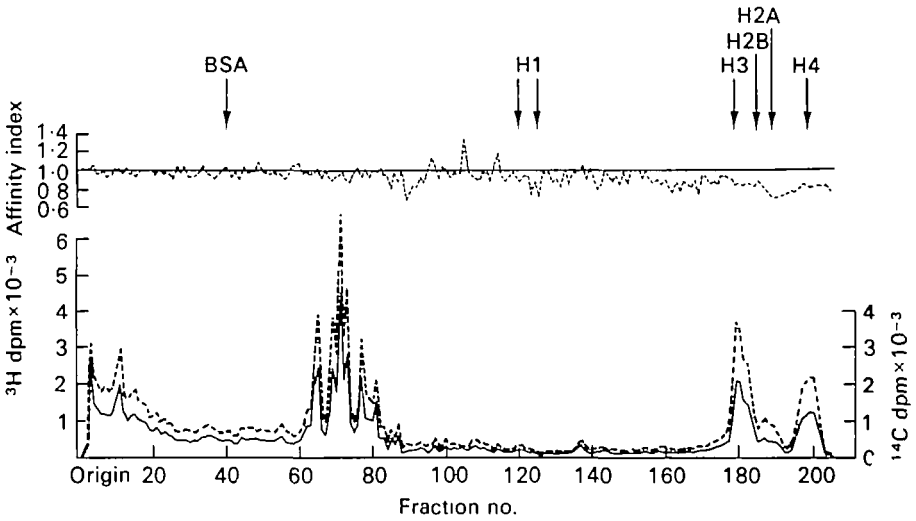

Fig. 9. A comparison of the proteins of unirradiated and γ-irradiated nucleoids isolated in 1·2 M NaCl. Cells labelled with [^{3}H]leucine or [^{14}C]leucine were applied to 2 sucrose gradients containing 1·2 M NaCl but no ethidium. The gradient containing ^{3}H-labelled cells was γ-irradiated and 15 min after the addition of the cells to the gradients, the gradients were spun at 5500 rev/min for 25 min. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2. — —, ^{3}H dpm from irradiated nucleoids; ——, ^{14}C dpm from unirradiated nucleoids; – –, affinity index of the proteins in each gel slice.

The released energy is available to assist binding so that at low concentrations ethidium binds more tightly to supercoiled DNA than to its relaxed counterpart (Bauer & Vinograd, 1968, 1971; Davidson, 1972; Hsieh & Wang, 1975). Histones H2A and H2B behave like ethidium in their binding to DNA. (We have revealed a differential affinity of these histones for DNA under dissociating conditions; we assume that the differences remain at equilibrium.) They might transmute free energy of supercoiling into binding energy by intercalating into or by melting or denaturing the DNA or by folding it into a left-handed toroidal superhelix (Cook & Brazell, 1977). The latter is consistent with observations that histones unwind and condense supercoiled circles of SV_{40} DNA without nicking them (Griffith, 1975; Germond *et al.* 1975). When histones dissociate from constrained DNA during the formation of nucleoids they leave residual imprints – the supercoils – in the DNA. SBP1, like H2A and H2B, binds more tightly to unirradiated nucleoids and so might also stabilize supercoiling. It remains to be seen whether it is related to other 'unwinding' proteins (for reviews see Herrick & Alberts, 1976*a*, *b*; Herrick, Delius & Alberts, 1976).

Our study shows that H3 and H4 do not bind more avidly to unirradiated nucleoids so that they may not stabilize supercoiling *in vivo*. On the other hand, they may stabilize supercoils but the earlier dissociation of H2A and H2B might generate all the potentially available free energy of supercoiling: dissociation of H3 and H4 at higher salt concentrations could not then generate further free energy of supercoiling. If so, our results need not conflict with those suggesting that H3 and H4, but not H2A

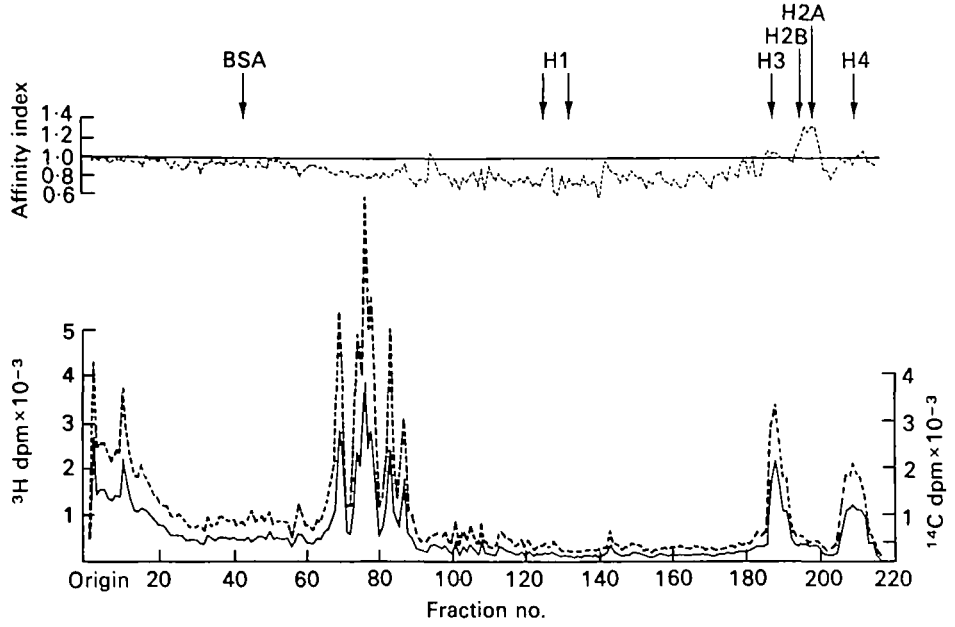

Fig. 10. The effect of ethidium on the protein content of unirradiated nucleoids isolated in 1·2 M NaCl. Cells labelled with [^{14}C]leucine were applied to a sucrose gradient containing 1·2 M NaCl but lacking ethidium. Others, labelled with [^{3}H]leucine, were layered on a similar gradient containing 16 μg/ml ethidium. Neither gradient was irradiated. Gradients were spun at 5500 rev/min for 25 min. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2. — —, ^{3}H dpm from nucleoids isolated in the presence of ethidium; ——, ^{14}C dpm from nucleoids isolated in the absence of ethidium; – –, affinity index of the proteins in each slice of the gel.

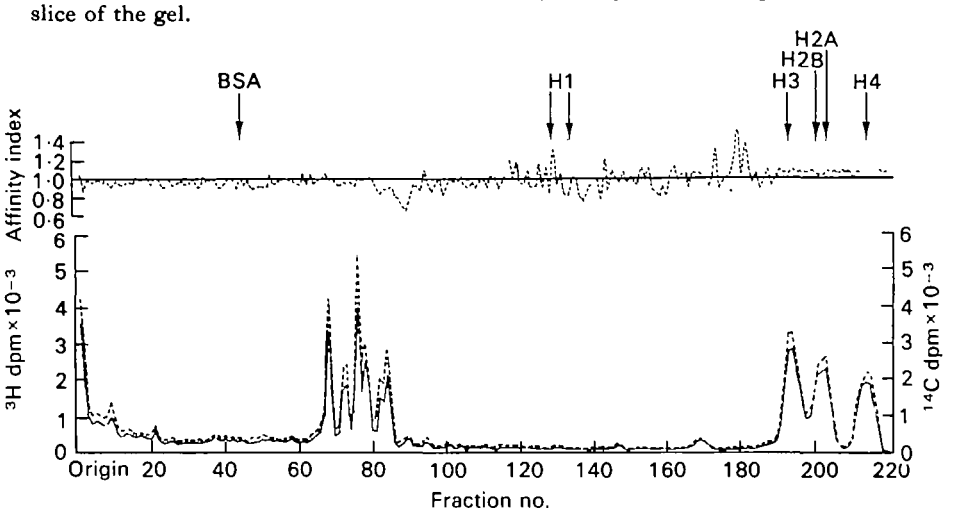

Fig. 11. Labelling with [^{14}C]leucine or [^{3}H]leucine does not affect the protein content of nucleoids. Cells labelled with [^{3}H]leucine or [^{14}C]leucine were applied to 2 identical sucrose gradients containing 1·0 M NaCl but lacking ethidium (see legend to Fig. 6). Neither gradient was irradiated. Nucleoids were isolated and their proteins analysed as described in the legend to Fig. 2. — —, ^{3}H dpm from unirradiated nucleoids; ——, ^{14}C dpm from unirradiated nucleoids; – –, affinity index of the proteins in each gel slice.

and H2B, are involved in the primary folding of DNA in chromatin (Wilkins *et al.* 1959; Richards & Pardon, 1970; Griffith, 1975; Boseley *et al.* 1976; Solner-Webb, Camerino-Otero & Felsenfeld, 1976).

When the RNA polymerase of *Escherichia coli* binds to DNA the double helix is unwound (Saucier & Wang, 1972) so that free energy of supercoiling is transmuted into binding energy. For this reason supercoiling in nucleoid DNA influences the initiation of RNA synthesis as it does in other templates (Colman & Cook, 1977). *In vivo* polymerase could only utilize the free-energy of supercoiling if the histone-DNA complex were disrupted, for example, by replacing the histones in the nucleosome and so maintaining DNA condensation or by melting (i.e. unwinding) and so extending the DNA. Dissociation of histones from an unconstrained DNA will yield no free energy of supercoiling to assist polymerase binding and this lack may underlie genetic inactivity (Cook & Brazell, 1976*a*). Similarly, differences in DNA folding by histones or non-histone proteins or even in the spacings of histones along the DNA (Compton, Bellard & Chambon, 1976) might influence RNA synthesis through effects on the free-energy of supercoiling (Cook, 1973, 1974).

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